09/889314

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

REQUEST FOR FILING NATIONAL PHASE PCT APPLICATION UNDER 35 U.S.C. 371 AND 37 CFR 1.494 OR 1.495

JC18 Rec'd PCT/PTO 16 JUL 2001

To: Hon. Commissioner of Patents
Washington, D.C. 20231

00909

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US)

Atty Dkt: P 0281578 /M99/0035/US
M# /Client Ref.

From: Pillsbury Winthrop LLP, IP Group:

Date: July 16, 2001

This is a **REQUEST** for **FILING** a PCT/USA National Phase Application based on:

1. International Application	2. International Filing Date	3. Earliest Priority Date Claimed
PCT/GB00/00237	28 January 2000	5 February 1999
⇧ country code	Day MONTH Year	Day MONTH Year (use item 2 if no earlier priority)

4. Measured from the earliest priority date in item 3, this PCT/USA National Phase Application Request is being filed within:

(a) ☐ 20 months from above item 3 date (b) ☒ 30 months from above item 3 date,

(c) Therefore, the due date (unextendable) is August 5, 2001

5. Title of Invention MEDICAMENT

6. Inventor(s) BURNIE, James Peter et al

Applicant herewith submits the following under 35 U.S.C. 371 to effect filing:

7. ☒ Please immediately start national examination procedures (35 U.S.C. 371 (f)).

8. ☒ **A copy of the International Application** as filed (35 U.S.C. 371(c)(2)) is transmitted herewith (file if in English but, if in foreign language, file only if not transmitted to PTO by the International Bureau) including:

a. ☐ Request;
b. ☒ Abstract;
c. 24 pgs. Spec. and Claims;
d. _____ sheet(s) Drawing which are ☐ informal ☐ formal of size ☐ A4 ☐ 11"

9. ☒ **A copy of the International Application has been transmitted by the International Bureau.**

10. **A translation of the International Application** into English (35 U.S.C. 371(c)(2))

a. ☐ is transmitted herewith including: (1) ☐ Request; (2) ☐ Abstract;
(3) _____ pgs. Spec. and Claims;
(4) _____ sheet(s) Drawing which are:
☐ informal ☐ formal of size ☐ A4 ☐ 11"

b. ☐ is not required, as the application was filed in English.

c. ☐ is not herewith, but will be filed when required by the forthcoming PTO Missing Requirements Notice per Rule 494(c) if box 4(a) is X'd or Rule 495(c) if box 4(b) is X'd.

d. ☐ Translation verification attached (not required now).

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11. ☒ Please see the attached Preliminary Amendment

12. ☐ Amendments to the claims of the International Application **under PCT Article 19 (35 U.S.C. 371(c)(3)), i.e., before 18th month from first priority date above in item 3, are transmitted herewith (file only if in English) including:**

13. ☒ PCT Article 19 claim amendments (if any) have been transmitted by the International Bureau

14. ☐ Translation of the amendments to the claims **under PCT Article 19 (35 U.S.C. 371(c)(3)), i.e., of claim amendments** made before 18th month, **is attached (required by 20th month from the date in item 3 if box 4(a) above is X'd, or 30th month if box 4(b) is X'd, or else amendments will be considered canceled).**

15. **A declaration of the inventor** (35 U.S.C. 371(c)(4))
a. ☐ is submitted herewith ☐ Original ☐ Facsimile/Copy
b. ☒ is not herewith, but will be filed when required by the forthcoming PTO Missing Requirements Notice per Rule 494(c) if box 4(a) is X'd or Rule 495(c) if box 4(b) is X'd.

16. **An International Search Report (ISR):**
a. Was prepared by ☒ European Patent Office ☐ Japanese Patent Office ☐ Other
b. ☒ has been transmitted by the international Bureau to PTO.
c. ☒ copy herewith (2 pg(s).) ☒ plus Annex of family members (1 pg(s).).

17. **International Preliminary Examination Report** (IPER):
a. ☒ has been transmitted (if this letter is filed after 28 months from date in item 3) in English by the International Bureau with Annexes (if any) in original language.
b. ☒ copy herewith in English.
c.1 ☐ IPER Annex(es) in original language ("Annexes" are amendments made to claims/spec/drawings during Examination) including attached amended:
c.2 ☐ Specification/claim pages #_____ claims #_____
Dwg Sheets #_____
d. ☐ Translation of Annex(es) to IPER **(required by 30th month due date, or else annexed amendments will be considered canceled).**

18. **Information Disclosure Statement** including:
a. ☒ Attached Form PTO-1449 listing documents
b. ☒ Attached copies of documents listed on Form PTO-1449
c. ☒ A concise explanation of relevance of ISR references is given in the ISR.

19. ☐ **Assignment** document and Cover Sheet for recording are attached. Please mail the recorded assignment document back to the person whose signature, name and address appear at the end of this letter.

20. ☐ Copy of Power to IA agent.

21. ☐ **Drawings** (complete only if 8d or 10a(4) not completed): ___ sheet(s) per set: ☐ 1 set informal; ☐ Formal of size ☐ A4 ☐ 11"

22. Small Entity Status ☐ is **Not** claimed ☒ is claimed (**pre**-filing confirmation required)
22(a) _____ (No.) Small Entity Statement(s) enclosed (since 9/8/00 Small Entity Statements(s) not essential to make claim)

23. **Priority** is hereby claimed under 35 U.S.C. 119/365 based on the priority claim and the certified copy, both filed in the International Application during the international stage based on the filing in (country) GREAT BRITAIN of:

	Application No.	Filing Date		Application No.	Filing Date
(1)	9902555.3	February 5, 1999	(2)		
(3)			(4)		
(5)			(6)		

a. ☒ See Form PCT/IB/304 sent to US/DO with copy of priority documents. If copy has not been received, please proceed promptly to obtain same from the IB.
b. ☒ Copy of Form PCT/IB/304 attached.

RE: USA National Phase Filing of PCT/GB00/00237

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24. Attached: 9 pages of Sequence Listiing

25 Per Item 17.c2, **cancel original** pages #_____, claims #_____, Drawing Sheets #_____

26. **Calculation of the U.S. National Fee (35 U.S.C. 371 (c)(1)) and other fees is as follows:**
Based on amended claim(s) per above item(s) ☐ 12, ☐ 14, ☐ 17, ☐ 25 (hilite)

Total Effective Claims	minus 20 =	x $18/$9 =	$0	966/967
Independent Claims	minus 3 =	x $80/$40 =	$0	964/965
If any proper (ignore improper) Multiple Dependent claim is present,		add$270/$135	+0	968/969

BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(4)): →→ **BASIC FEE REQUIRED, NOW** →→→→

A. If country code letters in item 1 are **not** "US","BR","BB","TT","MX","IL" "NZ", "IN" or "ZA"

See item 16 re:

1. Search Report was not prepared by EPO or JPO - - - - - - - - -	add$1000/$500		960/961
2. Search Report was prepared by EPO or JPO - - - - - - - - - - - -	add$860/$430	+430	970/971

SKIP B, C, D AND E UNLESS country code letters in item 1 are "US","BR","BB","TT","MX","IL", "NZ", "IN" or "ZA"

→ ☐ B. If USPTO did not issue both International Search Report (ISR) and (if box 4(b) above is X'd) the International Examination Report (IPER), -	add$1000/$500	+0	960/961
(only) (one) → ☐ C. If USPTO issued ISR but not IPER (or box 4(a) above is X'd), -	add$710/$355	+0	958/959
(these) (4) → ☐ D. If USPTO issued IPER but IPER Sec. V boxes not all 3 (boxes) YES, -	add$690/$345	+0	956/957
→ ☐ E. If international preliminary examination fee was paid to USPTO and Rules 492(a)(4) and 496(b) satisfied (IPER Sec. V all 3 boxes YES for all claims), - - - - - - - - - - - - -	add $100/$50	+0	962/963
27.	**SUBTOTAL =**	$430	
28. If Assignment box 19 above is X'd, add Assignment Recording fee of ----$40		+0	(581)
29. Attached is a check to cover the -	**TOTAL FEES**	$430	

Our Deposit Account No. 03-3975
Our Order No. 050885 (C#) | 0281578 (M#)

00909

CHARGE STATEMENT: The Commissioner is hereby authorized to charge any fee specifically authorized hereafter, or any missing or insufficient fee(s) filed, or asserted to be filed, or which should have been filed herewith or concerning any paper filed hereafter, and which may be required under Rules 16-18 and 492 (missing or insufficient fee only) now or hereafter relative to this application and the resulting Official document under Rule 20, or credit any overpayment, to our Account/Order Nos. shown above for which purpose a duplicate copy of this sheet is attached.
This CHARGE STATEMENT does not authorize charge of the issue fee until/unless an issue fee transmittal form is filed

Pillsbury Winthrop LLP
Intellectual Property Group

By Atty: Paul N. Kokulis **Reg. No.** 16773

Sig: _____ **Fax:** (703) 905-2500
Tel: (703) 905-2118

Atty/Sec: PNK/mhn

NOTE: File in duplicate with 2 postcard receipts (PAT-103) & attachments.

Attorney Docket No.

Applicant or Patentee: James Peter BURNIE and Ruth Christine MATTHEWS
International Appl. No.: PCT/GB00/00237
Filed: 28 January 2000
For: **Medicament**

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(f) and 1.27(b)) INDEPENDENT INVENTOR

As a below named inventor, I hereby declare that I qualify as an independent inventor as defined in 37 CFR 1.9 (c) for purposes of paying reduced fees under section 41 (a) and (b) of Title 35, United States Code, to the Patent and Trademark Office with regard to the invention entitled described in:

[ ] the specification filed herewith
[X] International application no. PCT/GB00/00237
[X] filed 28 January 2000

I have not assigned, granted, conveyed or licensed except as shown in the attachment hereto and am under no obligation under contract or law to assign, grant, convey or license, any rights in the invention to any person who could not be classified as an independent inventor under 37 CFR 1.9 (c) if that person had made the invention, or to any concern which would not qualify as a small business concern under 37 CFR 1.9 (d) or a nonprofit organization under 37 CFR 1.9 (e).

Each person, concern or organization to which I have assigned, granted, conveyed, or licensed or am under an obligation under contract or law to assign, grant, convey, or license any rights in the invention is listed below:

[ ] no such person, concern, or organization
[X] persons, concerns or organizations listed below*

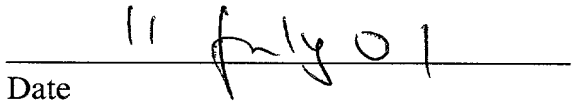
FULL NAME: NeuTec Pharma plc
ADDRESS: St James's Court
Brown Street
Manchester
M2 2JT Great Britain

[ ] Individual [X] Small Business Concern [ ] Nonprofit Organization

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28 (b))

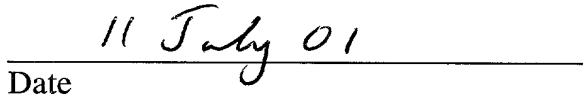
I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

James Peter BURNIE



Date

Ruth Christine MATTHEWS



Date

Attorney Docket No.

Applicant or Patentee	James Peter BURNIE and Ruth Christine MATTHEWS
Serial or Patent No.:	PCT/GB00/00237
Filed or Issued:	20 January 2000
Title:	Medicament

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS [37 CFR 1.9 (f) AND 1.27 (c)] - SMALL BUSINESS CONCERN

I hereby declare that I am

[X] the owner of the small business concern identified below :
[ ] an official of the small business concern empowered to act on behalf of the concern identified below :

NAME OF CONCERN **NeuTec Pharma plc**

ADDRESS OF CONCERN **St James's Court**
Brown Street
Manchester
M2 2JT GB

I hereby declare that the above identified small business concern qualifies as a small business concern as defined in 13 CFR 121.3-18 and reproduced in 37 CFR 1.9(d), for purpose of paying reduced fees under Section 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties control or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention, entitled

Medicament

by inventors James Peter BURNIE and Ruth Christine MATTHEWS described in

[ ] the specification filed herewith
[X] the application identified above
[ ] Application Serial No. filed
[ ] Patent No. , issued

If the rights held by the above identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 CFR 1.9(d) or by any concern which would not qualify as a small business concern under

37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e). *Note: Separate verified statements are required from each named person, concern, or organization having rights to the invention averring to their status as small entities [37 CFR 1.27].

FULL NAME
ADDRESS
[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATION

FULL NAME
ADDRESS
[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this Application or Patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate [37 CFR 1.28(b)].

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING ......James S Burnie......

TITLE OF PERSON OTHER THAN OWNER ......Director......

ADDRESS OF PERSON SIGNING ......1 Greystoke Drive......
Alderley Edge Cheshire

SIGNATURE ......[signature]...... DATE ..11 July 01..

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re PATENT APPLICATION OF

Inventor(s): BURNIE, James Peter et al

Filed: Herewith

Title: MEDICAMENT

July 16, 2001

PRELIMINARY AMENDMENT

Hon. Commissioner of Patents
Washington, D.C. 20231

Sir:

Please amend this application as follows:

IN THE SPECIFICATION:

At the top of the first page, just under the title, insert

☒ --This application is the National Phase of International Application PCT/GB00/00237 filed January 28, 2000 which designated the U.S. and that International Application

☒ was ☐ was not published under PCT Article 21(2) in English.--

Respectfully submitted,

PILLSBURY WINTHROP LLP
Intellectual Property Group

By: _____

Attorney: Paul N. Kokulis
Reg. No: 16773
Tel. No.: (703) 905-2118
Fax No.: (705) 905-2500

Atty\Sec. PNK/mhn
1600 Tysons Boulevard

McLean, VA 22102
(703) 905-2000

SEQUENCE LISTING

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 65                  70                  75                  80

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Thr Pro Arg Ser Glu Val Ile Glu Ile Gly Leu Ala Leu Ala Lys Ala
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Ser Gly Lys Asp Lys Thr Ser Ser Thr Thr Lys Thr Glu Thr Ala Pro
65                  70                  75                  80
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Gly Ala Asp Thr Gly Val Ser Gly Ala Ala Ala Thr Thr Ala Ser Asn
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Thr Ala Thr Lys Ile Ala Met Gln Thr Ser Ile Glu Glu Ala Ser Lys
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Leu Val Lys Ala Ile Ala Lys Ala Ile Ser Lys Gly Ile Ser Lys Val
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Asn Ile Met Ser Gln Val Leu Thr Ser Thr Pro Gln Gly Val Pro Gln
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Gln Asp Lys Leu Ser Gly Asn Glu Thr Lys Gln Ile Gln Gln Thr Arg
 65                  70                  75                  80

Gln Gly Lys Asn Thr Glu Met Glu Ser Asp Ala Thr Ile Ala Gly Ala
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Thr Pro Arg Ser Glu Val Ile Glu Ile Gly Leu Ala Leu Ala Lys Ala
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Ile Gln Thr Leu Gly Glu Ala Thr Lys Ser Ala Leu Ser Asn Tyr Ala
225                 230                 235                 240

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APPLICATION UNDER UNITED STATES PATENT LAWS

Atty. Dkt. No. PW 0281578/M99/0035/US
(M#)

Invention: MEDICAMENT

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This is a:

- ☐ Provisional Application
- ☐ Regular Utility Application
- ☐ Continuing Application
  - ☒ The contents of the parent are incorporated by reference
- ☒ PCT National Phase Application
- ☐ Design Application
- ☐ Reissue Application
- ☐ Plant Application
- ☐ Substitute Specification
  Sub. Spec Filed _____
  in App. No. _____ / _____
- ☐ Marked up Specification re
  Sub. Spec. filed _____
  In App. No _____ / _____

SPECIFICATION

Medicament

The present invention concerns treatment, prevention and diagnosis of infection due to *Chlamydia pneumoniae* and in particular to the prevention and treatment of atherosclerosis, including coronary atherosclerosis, caused by same.

C. pneumoniae is associated with atherosclerosis but no definitive link between the two has yet been established (Hammerschlag, M.R., 1998, Eur. J. Clin. Microbiol. Infect. Dis., 17: 305-308). Friedank, H.M. *et al.* (1993, Eur. J. Clin. Microbiol. Infect. Dis., 12(12): 947-951) identify a 54 kDa *C. pneumoniae* antigen which was recognised by 93% of sera positive for *C. pneumoniae*, the antigen appearing to be located on the surface of elementary bodies. Wiedman, A.A.M. *et al.* (1997, Clin. Diagn. Labs. Immunol., 4(6):700-704) showed the infectivity of *C. pneumoniae* elementary bodies to be slightly reduced by the use of antibody specific against a 54 kDa *C. pneumoniae* protein.

Despite investigating it, other researchers have not confirmed the immunogenicity of the *C. pneumoniae* 54 kDa band (see for example Kutlin, A. and Roblin, P.M., 1998, J. Infect. Dis., 177: 720-724; Campbell, L.A. *et al.*, 1990, J. Clin. Microbiol., 28(6): 1261-1264; Campbell, L.A. *et al.*, 1990. Infection and Immunity, 58(1): 93-97; Puolakkainen, M. *et al.*, 1993, J. Clin. Microbiol., 31(8): 2212-2214; hkima, Y. *et al.*, 1994, J. Clin. Microbiol., 32(3): 583-588; Maass, M. and Gieffers, J., 1997, J. Infection, 35: 171-176; Gonen, R. *et al.*, 1993, APMIS, 101:719-726).

The present inventor has now succeeded in isolating, purifying and identifying a *C. pneumoniae* protein which (together with inhibitors of same, such as

antibodies) is protective and therapeutic against *C. pneumoniae* infection. The therapeutic role of the protein has previously neither been suggested nor disclosed.

According to the present invention there is provided a *C. pneumoniae* protein having the amino acid sequence of SEQ ID NO: 2, for use in a method of treatment or diagnosis of the human or animal body. The amino acid sequence has been confirmed by N-terminal amino-acid sequencing (see "Experimental" below) and the protein has a theoretical molecular weight of 50.8 kDa, although post-translational modifications such as glycosylation may of course affect its apparent molecular weight as determined by e.g. SDS-PAGE. Experiments (below) have shown it to have an apparent molecular weight of 51 kDa on SDS-PAGE gels.

As can be seen from the plethora of publications above, although some identify immunogenic bands at molecular weights of 50-54 kDa, no specific therapeutically effective proteins have been identified.

Experiments (below) have allowed the present inventor to isolate and purify the protein of the present invention and identify the gene sequence coding for the protein. This has allowed the determination of the protein amino acid sequence (above). The nucleotide sequence coding for same forms another part of the present invention. Thus according to the present invention there is also provided a nucleotide sequence coding for a protein according to the present invention, for use in a method of treatment or diagnosis of the human or animal body. Such a nucleotide sequence may have the sequence of SEQ ID NO: 1. Modified nucleotide sequences having codons encoding the same amino acid sequence will be readily apparent to one skilled in the art.

The nucleotide sequence of the present invention and the amino acid sequence it encodes are already known from the Chlamydia Genome Project

(*C. pneumoniae* CWL029/CPn0809), as is an apparent *C. trachomatis* homologue (CT578). However, therapeutic and diagnostic uses for same have not been previously suggested.

The invention also extends to encompass forms of the protein which have been insubstantially modified (i.e. which have been partially modified), particularly forms of the protein which display the same immunogenic properties as the protein itself.

By "partial modification" and "partially modified" is meant, with reference to amino acid sequences, a partially modified form of the molecule which retains substantially the properties of the molecule from which it is derived, although it may of course have additional functionality. Partial modification may, for example, be by way of addition, deletion or substitution of amino acid residues. Substitutions may be conserved substitutions. Hence the partially modified molecule may be a homologue of the molecules from which it was derived. It may, for example, have at least 70% homology with the molecule from which it was derived. It may for example have at least 80, 90 or 95% homology with the molecule from which it was derived. An example of a homologue is an allelic mutant.

Also provided according to the present invention is the use of a protein, immunogenic fragment thereof or nucleic acid sequence encoding same according to the present invention in the manufacture of a medicament for the treatment of infection due to *C. pneumoniae*.

Immunogenic fragments of the protein include any fragment of the protein which elicits an immune response, and includes epitopes. Analogues (mimotopes) of epitopes may be readily created, the mimotopes having different sequences but displaying the same epitope and thus the term "immunogenic fragments" also

encompasses immunogenic analogues of the fragments e.g. mimotopes. Epitopes may be readily determined and mimotopes readily designed (Geysen, H.M. *et al.*, 1987, Journal of Immunological Methods, 102: 259-274; Geysen, H.M. *et al.*,1988, J. Mol. Recognit., 1(1):32-41; Jung, G. and Beck-Sickinger, A.G., 1992, Angew. Chem. Int. Ed. Eng., 31: 367-486). Such an immunogenic fragment carrying epitopes may also be described as being a peptide having the amino acid sequence of the immunogenic fragment and which carries an epitope.

The present inventor has succeeded in isolating a number of epitopes (immunogenic fragments) of the protein of the present invention. Thus according to the present invention there is also provided an epitope having the amino acid sequence of any one of SEQ ID NOs: 4-14. In particular, SEQ ID NOs: 5-7 provide an overlapping set of highly immunogenic peptides - as can be seen from the experimental data (below) SEQ ID NO: 5 provides for especially good results. Similarly, excellent results are also obtained from SEQ ID NO: 8.

The protein, immunogenic fragments thereof and nucleic acid sequences encoding same may be used in therapy, both prophylactically (e.g. as immunostimulants such as vaccines) and for treatment of infection due to *C. pneumoniae*. For example a nucleotide sequence encoding the protein or immunogenic fragment thereof may be used in the manufacture of a DNA vaccine (Montgomery, D.L. *et al.*, 1997, Pharmacol. Ther., 74(2): 195-205; Donnelly, J.J. *et al.*, 1997, Annu. Rev. Immunol., 15: 617-648; Manickan, E. *et al.*, 1997, Crit. Rev. Immunol., 17(2): 139-154).

Binding agents and inhibitors (such as antibodies or other neutralising agents) specific against the protein and immunogenic fragments thereof may also be used both diagnostically and therapeutically. Binding agents have a target to which they are specific, and in the case of a binding agent being an antibody, the target is an antigen.

An example of a therapeutic medicament is antibody specific against the protein of the present invention, and this may be employed in immunotherapy, for example passive immunotherapy. Antibodies, their manufacture and use are well known (Harlow, E. and Lane, D., "Using Antibodies - A Laboratory Manual", Cold Spring Harbor Laboratory Press, New York, 1998) and so antibodies and antigen binding fragments thereof will be readily apparent to one skilled in the art, and reference herein to antibodies is also reference to antigen binding fragments unless stated otherwise. Other inhibitors such as ribozymes, antisense oligonucleotides and DNA vaccines will be readily apparent to one skilled in the art (Fries, P.C., 1999, "DNA Vaccines", New England Journal of medicine, 341: 1623-1624; Leitner, W.W. *et al.*, 1999, "DNA and RNA based vaccines: principles, progress and prospects", Vaccine, 18: 765-777; Muotri, A.R. et al., 1999, "Ribozymes and the anti-gene therapy: how a catalytic RNA can be used to inhibit gene function", Gene, 237: 303-310; Rossi, J.J., 1999, "Ribozymes, genomics and therapeutics", Chemistry & Biology, 6: R33-R37; James, H.A., 1999, "The potential application of ribozymes for the treatment of haematological disorders", Journal of Leukocyte Biolofy, 66: 361-368)

Thus the present invention also provides the use of a inhibitor specific to the protein of the present invention in the manufacture of a medicament for the treatment of infection due to *C.pneumoniae.*

Also provided according to the present invention is a method of manufacture of a medicament for the treatment of infection due to *C. pneumoniae*, characterised in the use of a protein, immunogenic fragment or inhibitor according to the present invention.

Also provided according to the present invention is a method of treatment of infection due to *C. pneumoniae*(e.g. of a patient in need of same), comprising the step

of administering to a patient a medicament comprising a protein, immunogenic fragment or inhibitor according to the present invention. The exact dose of medicament administered to a patient may be readily determined using simple dose-response assays. Medicaments may additionally comprise a pharmaceutically acceptable carrier, diluent or excipient (Remington's Pharmaceutical Sciences and US Pharmacopeia, 1984, Mack Publishing Company, Easton, PA, USA)

It has not been previously suggested that the protein of the present invention (or immunogenic fragments of same) is diagnostic for infection due to *C. pneumonia.* Binding agents specific to the protein of the present invention (for example antibodies) may also be used diagnostically, for example in an ELISA-type test. Thus also provided according to the present invention is the use of a protein, immunogenic fragment or binding agent according to the present invention in the manufacture of a diagnostic test for *C. pneumoniae.*

Also provided is a diagnostic test method for infection due to *C. pneumoniae* comprising the steps of:

I) reacting an antibody specific against the protein of the present invention with serum from a patient;

ii) detecting an antibody-antigen binding reaction; and

iii) correlating the detection of an antibody-antigen binding reaction with the presence of the protein.

Such test methods may also be performed using other binding agents specific to the protein of the present invention.

Also provided is a kit of parts for performing such a test, characterised in that it comprises antibody specific against the protein of the present invention.

The invention will be further apparent from the following description, with reference to the several figures of the accompanying drawings, which show, by way of example only, uses of the proteins of the present invention.

EXPERIMENTAL

The experiments below detail the identification of a number of peptides and antisera against same which are useful in the therapy and diagnosis of infections due to *Chlamydia pneumoniae*. Starting with sera from infected patients, blotting against clinical isolates of *Chlamydia pneumoniae* showed the presence of an immunodominant antigen with an apparent molecular weight of 51 kDa, the antigen being stable to and released by octylglucoside treatment. N-terminal amino acid sequencing of the protein of the 51 kDa band allowed sequence database probing, in turn identifying a *C. pneumoniae* protein and a *C. trachomatis* homologue. Epitope mapping allowed the identification of antigenic peptides, which together with antibody against them were tested for their therapeutic and diagnostic efficacy.

Western Blotting - Using the Novex nuPAGE Electrophoresis System.

1. SDS PAGE

Preparation of Sample:

1. 100 µl of Novex SDS Sample loading buffer was added to 400 µl of a preparation of a *Chlamydia pneumoniae* clinical isolate and the mixture placed into a boiling waterbath for 10 minutes.

2. 10 µl of the mixture was loaded into each well of a Novex 4-12% Bis-Tris NuPage gel (1.0 mm, 12 well). In addition, 4 µl of Novex Multimark molecular weight standards were added to a single well on each gel.

3. Electrophoresis was performed using 1x Novex MOPS electrophoresis buffer at 200v for 40 minutes.

Western Transfer Protocol:

1. The blotting apparatus and the gel membrane "sandwiches" were assembled according to the protocol described in the Novex instruction booklet provided with the gels.

2. Blotting was performed using 1x Novex Transfer buffer containing 20% methanol. Transfer was carried out at 30v (constant) for 1 hour.

3. Following transfer, the membranes were removed from the apparatus and left to "Block" overnight in 3% Bovine Serum Albumin (BSA) at 4 °C.

Probing With Patient's Serum:

1. The membranes were cut into strips and placed into the wells of incubation trays. Patients' serum was diluted 1 in 20 in 3% BSA and 2 ml added to each strip. (2 strips per patient).

2. The membranes were incubated at room temperature for 2 hours with agitation.

3. The strips were washed 5 times over 30 minutes with 0.85% NaCl/0.01% Tween 20.

4. 2 ml of goat anti-human IgM or IgG alkaline phosphatase conjugated anti-immunoglobulin diluted 1 in 4000 in 3% BSA were added to each strip. The strips were incubated for a further hour at room temperature with agitation.

5. The membranes were washed a further 5 times as previously described.

6. Antibody-antigen interaction was visualised by the addition of NBT/BCIP (50 mg/ml) in pH 9.5 phosphate buffer.

7. The reaction was allowed to proceed until the bands had reached the required intensity.

Sera

Group A: Children with respiratory tract infection and no evidence of *Chlamydia pneumoniae* as shown by negative microimmunofluorescence (less than 1 in 64) test (n=19).

Group B: Children with respiratory tract infection and a microimmunofluorescence titre greater than 1 in 512 (n=18).

Group C: Patients undergoing cardiac surgery for advanced coronary disease (n=32). Ten of these had antibody on immunoblot.

Group D: Adults with respiratory tract infection and a chlamydia complement fixation test greater than 1 in 40 (n=27) using LGV 2 as an antigen.

Group E: Adults with pelvic inflammatory disease due to *Chlamydia trachomatis* (n=21).

Group F: Sera (n=11) which were positive for the 60/62 kDa doublet and band at 51 kDa were retested on antigen prepared from *Chlamydia pneumoniae* where the purified elementary bodies were incubated with 1% octylglucoside at 37 °C for 30 minutes rather than in SDS.

Results:

Results of the sera blotting experiments are shown in Table 1. It should be noted that sera blotting determines the presence in patients of antibodies specific against a given antigen, and so when a patient has previously been infected by a pathogen and developed an immune response against an antigen, that immune response may still be detectable at a later date when the patient is no longer infected. Hence background results must be interpreted in light of the general infection of a population by the pathogen. For example, the general population has an infection rate by adulthood of approximately 10% for *C. pneumoniae*, thus a background rate of detection of *C. pneumoniae* antigens of up to 10% should be expected.

Conclusions:

The sera from Group A children did not recognise *C.pneumoniae* on immunoblot. The Group B sera from children with evidence of *C.pneumoniae* infection recognised a range of antigens with apparent molecular weights ranging from 30 to 180 kDa. IgM for an antigen complex at 60/62 kDa which occurred as a doublet was immunodominant as well as an antigen at 51 kDa. For IgG the antibody was most pronounced for the antigen at 51 kDa. In the cardiac patients, 23 produced antibody and this was for IgM against the bands at 67, 60/62 and 51 kDa. For IgG this was the band at 51 kDa. For Group D IgM was most pronounced for the 60/62 kDa doublet and IgG for the band at 180 kDa and the doublet at 60/62 kDa. This group of sera contains those with infection most likely due to *Chlamydia psittaci*. The sera from Group E patients infected with *Chlamydia trachomatis* did not cross-react.

Group F Sera

On re-blotting with those sera previously positive for the 60/62 kDa doublet and 51 kDa, the doublet disappeared whilst the band at 51 kDa remained. This showed that the band at 51 kDa was stable to and released by octylglucoside treatment.

Solubility in Octylglucoside

Using samples from Group F patients, separation of antigens from elementary bodies using 1-D gel electrophoresis and SDS gave a different staining pattern compared to using 1-D gel electrophoresis and octylglucoside. The 51 kDa band was still visible after octylglucoside. The pair of antigenic bands at 60/62 kDa was not visible in octylglucoside. Therefore a distinguishing character of the 51 kDa antigen of the present invention is its solubility in octylglucoside.

N-Terminal Amino Acid Sequencing

N-Terminal amino-acid sequencing was performed upon the 51 kDa band. The resulting sequence was then used to query the Chlamydia Genome Project database which identified the protein of SEQ ID NO: 2 and a *C. trachomatis* homologue.

Epitope Mapping

A series of overlapping peptides of 15 amino acids covering the derived amino acid sequence of the protein were synthesised on polyethylene pins with reagents from an epitope scanning kit (Cambridge Research Biochemicals, Cambridge, UK) as described previously by Geysen *et al.* (1987, Journal of Immunological Methods, 102: 259-274). Peptide 1 consisted of residues 1 to 15, peptide 2 consisted of residues 2 to 16 etc. The reactivity of each peptide with patient sera (diluted 1:200) was determined for IgG by ELISA. Data were expressed as A405 after 30 minutes of incubation.

Sera from patients as follows:

Group 1: Children with respiratory tract infection and no evidence of *Chlamydia pneumoniae* as shown by negative immunoblot and microimmunofluorescence (less than 1 in 64) (n = 3).

Group 2: Children with respiratory tract infection, positive immunoblot and microimmunofluorescence test greater than 1 in 512 (n = 6).

Group 3: Patients undergoing cardiac surgery for advanced coronary disease and antibody on immunoblot (n =2).

Group 4: Patients presenting with history of chest pain, negative troponin (<0.2), negative immunoblot (n = 3).

Group 5: Patients presenting with early coronary, positive troponin (>0.2) and antibody on immunoblot (n =8).

Results

Epitope mapping

Epitope mapping defined eleven areas where children with acute chlamydial infection produced wells with a mean optical density (OD) greater than 1. In the case of epitopes having SEQ ID NOs: 4, 5, 6, 7, 8, 10, 12 and 14 the mean OD was at least 2 standard deviations above that of Group 1 (children with no evidence of *C.pneumoniae* infections). This applied also to Groups 3, 4 and 5 with the exception of SEQ ID NO: 5 which was positive in Groups 4 and 5.

Peptide 1(SEQ ID NO: 15) representing epitope having the sequence of (i.e. which is carried by the peptides having the sequence of) SEQ ID NO: 8 and peptide 2 (SEQ ID NO: 16) representing the carboxy end of SEQ ID NO: 4, the epitope having the sequence of SEQ ID NO: 5 and the amino end of SEQ ID NO: 6 were synthesised.

Preparation of rabbit polyclonal serum

New Zealand white rabbits were pre-bled and then immunised subcutaneously with either peptide 1 or peptide 2 (0.1 ml of 1 mg/ml) conjugated to KLH suspended in either Freund's adjuvant (injection at day 0) or Freund's incomplete adjuvant on days 14, 42, and 70). Serum was obtained for indirect ELISA at the terminal bleed-out.

Indirect ELISA

By a simple adsorption of each peptide to a microtitre plate the following procedure was performed The peptide was dissolved in 2 ml of 0.01 M phosphate buffer saline (PBS), pH 7.2 and diluted to a concentration of 10 µg/ml (1/100) in the same buffer.

1. 150 µl aliquots of peptide (10 µg/ml in 0.01 M PBS) were pipetted into the wells of a Falcon 3912 microassay plate and were incubated overnight at 4 °C.
2. The unbound peptide was removed by washing four times (4 x 10 minutes) with 0.05% Tween 20 in 0.01 M PBS (pH 7.2).
3. The plates were blocked with 2% skimmed milk-10% FCS in 0.01 M PBS for 1 hour at 37 °C.
4. The plates were washed four times (4 x 10 minutes) with 0. 05% Tween 20 in 0.01 M PBS and the serum under investigation was added (1/100 dilution in blocking solution) into the wells of micro assay plate (three wells used for each serum) and incubated for 2 hours at 37 °C.
5. The plates were washed four times (4 x 10 minutes) with 0.05% Tween 20 in 0.01 M PBS and secondary antibody, anti-rabbit IgG peroxidase conjugate (1/1000 dilution in blocking solution) was added and incubation proceeded for 1 hour at 37 °C.
6. The plates were washed four times (4 x 10 minutes) with 0.05% Tween 20 in 0.01 M PBS, followed by a further washing with 0.01 M PBS. The plate was then incubated for 45 minutes at room temperature with agitation in 0.5

mg/ml of freshly prepared 2,2 Azino-bis [3-ethylbenz-thiazoline-6-sulfonic acid] diammonium (ABTS tablets) in pH 4.0 citrate buffer with 0.01% (w/v) hydrogen peroxide.

7. Optical density (OD) measurements were made with an ELISA plate reader (Titertek Miltiscan) at a wavelength of 405 nm.

8. The average readings for each three wells for each serum was determined.

Results

The results shown in Table 3 demonstrate seroconversion to each individual peptide.

Expression of the amino-end of the protein

The sequence was codon optimised (Genosys, California) for *E.coli* and a BamHI and Not1 site added to opposite ends. The optimised sequence and PET 29 vector (Novagen, Wisconsin) were restriction digested using BamHI and Not1 and transformed by heat shock into *E.coli* strain BL21 (Invitrogen, Carlsbad, California). The expressed amino acids were from amino acids 1-292 and included the epitopes represented by peptides 1 and 2. This construct included an S-tag and Thrombin cleavage site at the amino end and histidine tag at the carboxy end (SEQ ID NO: 3).

Purification

The transformants were expressed as follows. Briefly, 5 ml of an overnight culture was used to inoculate 500 ml LB (50 µg/ml kanamycin, 34 µg/ml chloramphenicol) which was grown for 2 hours at 37 °C to an OD 600 of 0.5, then induced for 3 hours with 0.1 mM IPTG (Sigma, Poole Dorset). The cells were pelleted and disrupted by crushing at -20 °C in an XPRESS. The buffer (50 mm NaH_2PO_4, 0.5 M NaCl, 10 mm imidazole) and the cell debris pelleted down. The supernatant was filter sterilised and put on a Ni-NTA agarose slurry affinity column (Qiagen) in order to capture the His-tagged recombinant protein. The column was washed 3 times with 4 ml of washing buffer and

the protein eluted maximally with 150 mM imidazole. The protein gave a single band on a 10% acrylamide gel stained with Coomassie Brilliant Blue with an apparent molecular weight of 37 kDa. On Western blot counterstaining with the anti-His mouse alkaline phosphate conjugate (1:2,500) (Sigma, Dorset, Poole) this produced a single band at 37 kDa and a breakdown product at 35 kDa. The protein concentration of the elute was measured and standardised to 10 mg/ml.

Amino acid sequencing

The protein was amino end cleared to remove the S-tag using a Thrombin cleavage Kit (Novagen). The digestion reaction was 5 µl 10 x Thrombin cleavage buffer, 0.5 mg purified recombinant protein, 1 µl of 0.01 µg/ml Thrombin which was left at room temperature for 18 hours. The reaction mix was run on a 12% SDS-PAGE gel and transferred onto PVDF membrane (Amersham, Chalfont, UK). This was stained with Coomassie Brilliant Blue and the protein bands destained and excised. Direct amino acid sequencing gave amino acids 28-32 of SEQ ID NO: 3 which matched the amino end (Department of Biochemistry, University of Cambridge).

Human recombinant antibodies

These peptides and the purified recombinant proteins were used to pan the phage display library. The peptide and recombinant protein were used at 10 mg/ml on NunC immunotubes Bst-N1 fingerprints of the PCR-amplified ScFv inserts before panning showed a highly heterogeneous library. After panning against peptide 1, 7 fingerprints were identified of which four were represented by more than one clone (A, B, C, D). These were combined as a pool for a neutralisation assay (pool 1) (below). After panning against peptide 2, clone A was present as well as a new ScFv, E. A and E were combined to produce pool 2. Against the clone recombinant fragment ScFvs E, F and G were present as well as a further ScFv, H. ScFvs E, F, G and H were tested together as pool 3.

Neutralisation assays

Chang cells (50 ml of 10^6 cells/ml) in maintenance media were grown overnight at 37 °C with 5% CO_2. Chang cells (1 ml of $1x10^6$ cells/ml maintenance media) were grown overnight at 37 °C with 5% CO_2 in plastic bijoux containing a thin glass circle on which the cells can grow. For recombinant protein or peptide assay (0.1 µl/ml), 100 µl of each sample was incubated with shaking for 1 hour with the cells at 37 °C. For the phage and sera assays, 100 µl of each sample (1:10 rabbit sera or dialysed phage pools 1-3) were incubated with 100 µl elementary bodies (EB) for 1 hour at 37 °C, shaking. After this first incubation, the 100 µl EB or 200 ml of the phage or rabbit sera/EB mix was added to the Chang cells. This was incubated with shaking for 1 hour at 37 °C. The supernatant was removed from every sample and replaced by 1 ml of fresh maintenance media. This was incubated at 37 °C with 5% CO_2 for 72 hours.

For both assays, the inclusion bodies were fixed and stained the following way; the cells were washed twice with PBS, then fixed with 100% methylated spirits for 10 minutes and washed twice again with PBS. The glass circles were incubated for 30 minutes with 10 µl of mouse *C.pneumoniae* inclusion bodies monoclonals (Mab) then washed 3 times with PBS and incubated for 30 minutes with 100 µl of fluorescein conjugated anti-mouse IgG. The inclusion bodies were then observed by fluorescence microscopy and three 200X fields counted. EB only samples were used as a positive control for chlamydial infection and dialysed phage supernatant without EB as a negative control.

Results

See Table 4 (Table of Neutralisation Assays).

Conclusion

Pre-incubation with the rabbit antiserum against peptide 2 and peptide 2 itself reduced the infectivity due to *C.pneumoniae*. Incubation with peptide 1 produced a similar reduction . The pools of phages were also active.

Overall this demonstrated the immunogenicity of the antigen, the potential therapeutic effect of peptides representing its key epitopes and both rabbit hyperimmune antiserum and ScFvs against these epitopes.

Table 1

Apparent Molecular Weight (kDa)	Group B (N=18)		Group C (N=18)		Group D (N=27)		Group E (N=21)	
	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG
180	1	2		2	1	6		1
130		2			1	4		
120	1	5		1	1	5		1
98		5		1	2	5		2
90		2				2		
67		2	5	1			1	1
60/62*	8	5	5		13	7	2	2
51	7	11	9	10	2	3	1	2
47	1	1	1		0	0	0	0
40	0	0	0	3	0	0	0	1
30		4	0	3		2		2

* runs as a doublet within 1 mm of each other

Table 2

		Value for [a]				
Well No.	Epitope SEQ ID NO	Group 1 (n = 3)	Group 2 (n = 6)	Group 3 (n = 2)	Group 4 (n = 3)	Group 5 (n = 8)
3	9	0.538±0.205	1.028±0.423	0.425±0.036	0.416±0.184	0.499±0.191
4		0.599±0.252	1.487±0.462	0.502±0.036	0.407±0.107	0.438±0.162
13	10	0.462±0.203	1.103±0.229	0.473±0.026	0.421±0.162	0.427±0.188
31	11	0.491±0.192	1.103±0.310	0.440±0.004	0.407±0.105	0.310±0.129
41	12	0.547±0.235	1.169±0.256	0.474±0.024	0.393±0.08	0.376±0.158
43	13	0.598±0.258	1.223±0.323	0.558±0.015	0.423±0.119	0.406±0.181
55	4	0.547±0.235	1.265±0.334	0.475±0.02	0.373±0.076	0.381±0.042
58	5	0.611±0.019	1.025±0.06	0.611±0.019	1.127±0.253	0.800±1.232
59	6	0.494±0.166	1.096±0.267	0.547±0.009	0.546±0.200	0.702±0.144
60	7	0.489±0.129	1.048±0.270	0.483±0.064	0.388±0.008	0.449±0.140
61		0.530±0.236	1.051±0.262	0.59±0.089	0.446±0.09	0.784±0.257
76	8	0.485±0.158	1.174±0.255	0.654±0.068	0.564±0.223	0.666±0.266
79	14	0.510±0.235	1.21±0.273	0.418±0.003	0.423±0.127	0.388±0.153

[a] Optical density ± Standard deviation

Table 3

	[a] Pre Serum	Post Serum
Peptide 1	0.055 ± 0.01	0.591 ± 0.06
Peptide 2	0.056 ± 0.01	0.507 ± 0.04

[a] optical density ± standard derivation

Table 4 - Table of Neutralisation Assays

	Number of Elementary Bodies in Three 200x Fields
Cell control (dialysed phage supernatant)	0
Cell control (elementary bodies)	30
Rabbit anti-serum	
Versus peptide 1	30
Versus peptide 2	19
Pre-incubation	
Peptide 1	13
Peptide 2	0
Recombinant protein	12
Phage Pools	
Pool 1	18
Pool 2	N/D
Pool 3	21

CLAIMS

1. A *C.pneumoniae* protein having the amino acid sequence of SEQ ID NO: 2 for use in a method of treatment or diagnosis of the human or animal body.

2. A nucleotide sequence encoding a protein according to claim 1 for use in a method of treatment of the human or animal body.

3. A nucleotide sequence according to claim 2, having the sequence of SEQ ID NO: 1.

4. The use of a protein, immunogenic fragment thereof or nucleotide sequence encoding same according to any one of the preceding claims in the manufacture of a medicament for the treatment of infection due to *C.pneumoniae*.

5. The use of an immunogenic fragment according to claim 4, having the amino acid sequence of any one of SEQ ID NOs: 4-14 in the manufacture of a medicament for the treatment of infection due to *C.pneumoniae*.

6. The use of an inhibitor specific against the protein, immunogenic fragment or nucleotide sequence encoding same according to any one of the preceding claims in a method of manufacture of a medicament for the treatment of infection due to *C.pneumoniae*.

7. The use of an inhibitor according to claim 6, the inhibitor being selected from the group of an antibody, DNA vaccine, ribozyme and antisense oligonucleotide.

8. A method of manufacture of a medicament for the treatment of infection by *C.pneumoniae* characterised in the use of a protein, immunogenic fragment thereof or nucleotide sequence encoding same according to either one of claims 4 or 5.

9. A method of manufacture of a medicament for the treatment of infection due to *C.pneumoniae* characterised in the use of an inhibitor according to either one of claims 6 or 7.

10. The use of a protein according to claim 1 or an immunogenic fragment thereof or a binding agent specific to same or an inhibitor of same in the manufacture of a diagnostic test for *C.pneumoniae*.

11. A kit of parts for a diagnostic test for *C.pneumoniae*, characterised in that it comprises a protein according to claim 1 or an immunogenic fragment thereof or a binding agent specific to same or an inhibitor of same.

12. A diagnostic test method for infection due to *C.pneumoniae* comprising the steps of:

i) reacting an antibody specific against the protein according to claim 1 with serum from a patient;

ii) detecting an antibody - antigen binding reaction; and

iii) correlating the detection of an antibody - antigen binding reaction with the presence of the protein.

13. A diagnostic test method according to claim 12, being a method of diagnosis of the human or animal body.

14. A method of treatment of infection due to *C.pneumoniae* comprising the step of administering to a patient a medicament comprising a protein, immunogenic fragment thereof, nucleotide sequence encoding same or an inhibitor thereof according to any one of claims 4-7.

FOR UTILITY/DESIGN CIP/PCT NATIONAL/PLANT ORIGINAL/SUBSTITUTE/SUPPLEMENTAL DECLARATIONS

RULE 63 (37 C.F.R. 1.63) DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

PW FORM

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name, and I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the **INVENTION ENTITLED Medicament**

the specification of which (CHECK applicable BOX(ES))

X BOX(ES) → A. ☐ is attached hereto.

→ B. ☐ was filed on _____ as U.S. Application No. _____ /

→ C. ☐ was filed as PCT International Application No. PCT/ GB00/00237 on 28 January 2000

and (if applicable to U.S. or PCT application) was amended on _____

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose all information known to me to be material to patentability as defined in 37 C.F.R. 1.56. Except as noted below, I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT International Application which designated at least one other country than the United States, listed below and have also identified below any foreign application for patent or inventor's certificate, or PCT International Application, filed by me or my assignee disclosing the subject matter claimed in this application and having a filing date (1) before that of the application on which priority is claimed, or (2) if no priority claimed, before the filing date of this application.

PRIOR FOREIGN APPLICATION(S)

Number	Country	Day/MONTH/Year Filed	Date first Laid-open or Published	Date Patented or Granted	Priority NOT Claimed
9902555.3	GB	05/02/1999			

If more prior foreign applications, X box *at bottom* and continue on attached page.

Except as noted below, I hereby claim domestic priority benefit under 35 U.S.C. 119(e) or 120 and/or 365(c) of the indicated United States applications listed below and PCT international applications listed above or below and, if this is a continuation-in-part (CIP) application, insofar as the subject matter disclosed and claimed in this application is in addition to that disclosed in such prior applications, I acknowledge the duty to disclose all information known to me to be material to patentability as defined in 37 C.F.R. 1.56 which became available between the filing date of each such prior application and the national or PCT international filing date of this application:

PRIOR U.S. PROVISIONAL, NONPROVISIONAL AND/OR PCT APPLICATION(S)

Application No. (series code/serial no.)	Day/MONTH/Year Filed	Status pending, abandoned, patented	Priority NOT Claimed

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

And I hereby appoint Pillsbury Winthrop LLP, Intellectual Property Group, telephone number (703) 905-2000 (to whom all communications are to be directed), and persons of that firm who are associated with USPTO Customer No 909 (see below label) individually and collectively my attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith and with the resulting patent, and I hereby authorize them to delete from that Customer No the names of persons no longer with their firm, to add new persons of their Firm to that Customer No, and to act and rely on instructions from and communicate directly with the person/assignee/attorney/firm/ organization who/which first sends/sent this case to them and by whom/which I hereby declare that I have consented after full disclosure to be represented unless/until I instruct the above Firm and/or an attorney of that Firm in writing to the contrary.

00909

USE ONLY FOR PILLSBURY WINTHROP

00909

(1) **INVENTOR'S SIGNATURE:** James P. Burnie **Date:** 11th July 2001

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☐ FOR ADDITIONAL INVENTORS see attached page.

☐ See additional foreign priorities on attached page (incorporated herein by reference).

Atty. Dkt. No. P _____ (M#)

Rule 56(a) & (b) = 37 C.F.R. 1.56(a) & (b)
PATENT AND TRADEMARK CASES - RULES OF PRACTICE
DUTY OF DISCLOSURE

(a) ...Each individual associated with the filing and prosecution of a patent application has a duty of candor and good faith in dealing with the [Patent and Trademark] Office, which includes a duty to disclose to the Office all information known to that individual to be material to patentability...(b) information is material to patentability when it is not cumulative and (1) It also establishes by itself, or in combination with other information, a prima facie case of unpatentability of a claim or (2) refutes, or is inconsistent with, a position the applicant takes in: (i) Opposing an argument of unpatentability relied on by the Office, or (ii) Asserting an argument of patentability

PATENT LAWS 35 U.S.C.

§102. Conditions for patentability; novelty and loss of right to patent

A person shall be entitled to a patent unless--

(a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for patent or

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of the application for patent in the United States, or

(c) he has abandoned the invention, or

(d) the invention was first patented or caused to be patented, or was the subject of an inventor's certificate, by the applicant or his legal representatives or assigns in a foreign country prior to the date of the application for patent in this country on an application for patent or inventor's certificate filed more than twelve months* before the filing of the application in the United States, or

(e) the invention was described in a patent granted on an application for patent by another filed in the United States before the invention thereof by the applicant for patent, or on an international application by another who has fulfilled the requirements of paragraphs (1), (2), and (4) of section 371(c) of this title before the invention thereof by the applicant for patent, or

(f) he did not himself invent the subject matter sought to be patented, or

(g) before the applicant's invention thereof the invention was made in this country by another who had not abandoned, suppressed, or concealed it. In determining priority of invention there shall be considered not only the respective dates of conception and reduction to practice of the invention, but also the reasonable diligence of one who was first to conceive and last to reduce to practice, from a time prior to conception by the other.

§103. Condition for patentability; non-obvious subject matter

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made. . . .

(c) Subject matter developed by another person, which qualified as prior art only under subsection (f) or (g) of section 102 of this title, shall not preclude patentability under this section where the subject matter and the claimed invention were, at the time the invention was made, owned by the same person or subject to an obligation of assignment to the same person.

* Six months for Design Applications (35 U.S.C. 172).

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